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Transformations of Potato Starch During Oxidation with Hypochlorite

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Five starch samples of different degree of oxidation were obtained with the help of sodium hypochlorite. Apart from that, commercial starch was divided into 3 fractions of differentiated size of grains and the fractions were also subjected to oxidation. The analysis of oxidized starch led to following results:

- large grains of starch undergo the oxidation process more easily than small grains, from oxidized starch hydrolysates glucuronic acid amounting to about 75% of carboxyl groups and gluconic acid to about 25% of carboxyl groups, can be separated;
- side reaction proceeding during oxidation process is hydrolytic degradation of starch molecules which is shown by the increase of reducing value and the decrease of viscosity of oxidized starch;
- in starch oxidized with hypochlorite absence of dialdehyde systems is characteristic in contrast to starch oxidized with periodate;
- in the process of oxidation with hypochlorite, starch molecules change their shape and spatial system, which is indicated by the changes of colour complexes with iodine, greater resistance to the action of amylolytic enzymes and greater capacity for forming flexible gels in the form of films.

Umwandlungen bei Kartoffelstärke während der Oxidation mit Hypochlorit. Durch Natriumhypochlorit-Oxidation wurden 5 Stärkeproben mit unterschiedlichem Oxidationsgrad erhalten. Gleichzeitig wurde handelsübliche Stärke in drei Fraktionen unterschiedlicher Korngröße aufgeteilt und die Fraktionen wurden ebenfalls der Oxidation unterworfen. Die Analyse der oxidierten Stärken führte zu folgenden Ergebnissen:

- große Stärkekörner werden dem Oxidationsprozeß leichter unterworfen als kleine Körner;
- aus den Hydrolysaten oxidiierter Stärke kann Glucuronsäure, entsprechend 75% der Carboxylgruppen, und Glukonsäure, entsprechend 25% der Carboxylgruppen, abgetrennt werden;
- die während des Oxidationsprozesses auftretende Nebenreaktion ist der hydrolytische Abbau der Stärkemoleküle, wie durch die Erhöhung des Reduktionswertes und die Erniedrigung der Viskosität oxidiierter Stärken gezeigt wird;
- für mit Hypochlorit oxidierte Stärke ist die Abwesenheit der für mit Periodat oxidierte Stärken typischen Dialdehydsysteme charakteristisch;
- während des Oxidationsprozesses mit Hypochlorit verändern die Stärkemoleküle ihr Form- und Raumsystem; dies wird aufgezeigt durch die Veränderungen der Farbkomplexe mit Iod, durch größere Resistenz gegenüber der Einwirkung amyolytischer Enzyme sowie durch die größere Resistenz gegenüber der Einwirkung amyolytischer Enzyme sowie durch die größere Fähigkeit zur Bildung flexibler Gele in Form von Filmen.

1 Introduction

Oxidation of starch is one of the most prevalent, beside acid and enzyme hydrolysis, methods of starch modification [8]. Among different means of chemical and physico-chemical modification, the process of starch oxidation is used to obtain particularly desired properties of starch preparations, useful for definite purposes. Starch preparations modified in the process of oxidation, are used in the food industry, textile industry, paper industry and in others. For nutritive purposes, the preparations of oxidized starch are obtained, as thickeners and subsidiary gelatine agents (sauces, jellies, puddings, ice-creams and the like).

The process of starch oxidation may proceed according to two types of reactions: the specific oxidation taking place under the influence of periodates and the reaction of unspecific oxidation taking place in the result of treating starch with various oxidizing agents such as: free halogens and their oxygen compounds, peroxides, nitrates, nitric oxides, persulphates, permanganates and so on. The specific oxidation of starch with the help of periodates is conditioned by production of a temporary complex combination between periodate

and the glycol system of carbohydrates, which causes the deformation (twist) of intercarbon bond in the glycol system and leads to bond cleavage forming two aldehyde groups with separated carbons. The mechanism of this specific reaction was described for the first time by Malaprade [12].

Sodium hypochlorite is the most frequently used oxidizing agent in the process of starch oxidation when realized in industrial practice [3, 4], more seldom, hypobromite, gaseous chlorine, chlorites and permanganates are used. As it is wellknown from fundamental theoretical considerations and numerous results of experimental studies [6, 7, 10, 14, 15, 16], during oxidation of carbohydrates the carbonyl group at anomer carbon is subjected the most easily to oxidation, the next is the hydroxyl group at carbon 6 (primary), the other hydroxyl groups at secondary carbons, that is 2, 3 and 4 are subjected to oxidation with considerable difficulty. Here, the doubts and dissent of different authors in the matter of effects of starch oxidation process by means of hypochlorite and other oxidants begin. These doubts and dissent are based on the lack of certainty whether the action of oxygen on starch by unspecific oxidants is limited only to the formation of carboxyl groups at anomer carbon and primary carbon, that

is the sixth, or whether it causes also formation of carbonyl groups at the second and the third carbons. The problem is complicated by the fact that owing to the low stability of glycosides bonds in starch, during the process of starch oxidation there occurs an intensive process of hydrolysis of the glycosides bonds, that it to say the degradation of polysaccharides molecules. In the result of this side reaction the latent and reducing carboxyl groups, situated at anomer carbons, are liberated. Formation, in starch molecules, of carboxyl groups (or gluconic and glucuronic acids) as well as the increase of the reducing capacity as indication of the formation of carboxyl groups and the degradation of polysaccharides molecules, is the effect of the process — consisting of two reactions (oxidation and hydrolysis) — of the action of an oxidizer on starch. Secondly, until now there has been no effective analytic way of distinguishing latent carbonyl groups at anomer carbons in starch from typical aldehyde groups which can possibly be found in the chains of the oxidized polysaccharides at carbons 2 and 3, as it takes place, after starch oxidation by periodate [19].

The aim of this paper is explanation of the doubts regarding the location of the functional groups undergoing oxidation in starch molecules, qualification of the proportions of the originating carboxyl and carbonyl groups, assignation of the influence of diversified size of grains on the course and the results of potato starch oxidation as well as qualification of practical effects and acquired properties of oxidized starch.

2 Analytic Methods

- Viscosity of 1% solution was determined with the help of Höppler's viscometer.
- Content of available chlorine was determined by iodometric method.
- Reducing value of starch or general content of carbonyl groups was determined by oximing method with hydroxylamine [11] and the result of this method was proved by comparison with the results obtained by the ferricyanide method. When it had been made certain that these two methods, in several sets, give nearly perfectly agreeing results, the further determinations were done by the oximing method as the considerably simpler one.
- The presence of typical aldehyde in the presence of the reducing saccharides was determined by a specially worked out colorimetric method, on the base of the specific aldehydes reaction with *Schiff's* reagent (fuchsinsulfurous acid). On the base of the very old *Schiff's* works [17] it is known that fuchsinsulfurous acid gives a colour reaction in the acid medium with aldehydes and it does not give such a reaction with reducing saccharides.
- The content of carboxyl groups was determined by the method based on titration of a hydrogen starch sample (obtained from washing of the starch with hydrochloric acid) solution NaOH and removing the content of acid groups originating from amylophosphoric acid, determined by the gravimetric-molybdenous method.
- The graininess of starch was defined by two methods: by microscopic measurements and with the help of sedimentation scale "Sartorius".
- The photometric determination of iodine adsorption by oxidized starches [1, 9] was done with the help of the photometer "Specol" in the solution containing 4 mg of starch in 100 ml when using 0.01 mol of iodine solution.
- Chromatographic analysis of hydrolysis products of the oxidized starch. (The conditions of the paper chromatography analysis: Whatman 1, developing solution propanol:water:ethyl acetate 6:3:1).

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- Saccharides were analyzed on paper chromatograms directly from the solution of hydrolysates.
- The presence of gluconic and glucuronic acids was determined chromatographically by binding them on the bed of mixed ionites and eluting them in this way from the hydrolysates of the oxidized starch; the solution of these acids after elution from the ionic column was analyzed by paper chromatography using the identical technique as on saccharides, on condition that the localization of spots of glucuronic acid on the paper was done after developing the chromatograms with *Buchan-Savage* reagent, while the identification of spots of gluconic acid was done after developing the chromatograms with *Taschiro* reagent as sodium gluconate, giving alkaline reaction and by that the green spots on the pink background of the chromatogram, measurable with the help of a planimeter.
- The content of glucuronic acid in oxidized starch hydrolysates beside the chromatographic determination, was determined by *Schoorl's* method directly in solution eluted from bed of mixed ionites after ion-exchange of definite sample of oxidized starch hydrolysate.
- External changes of starch granules were observed with the help of an optical microscope in plain and polarized light and also with the help of a scanning electron microscope, type ISM-50A, Jeol's firm [16].

3 Experiments

The process of starch oxidation by sodium hypochlorite was made in a glass reactor (Fig. 1) of 3 dm³ capacity, water-cooled. Inside of the reactor there were put the reagents as well as a stirrer (4), a thermometer (3), electrodes of the pH-meter and the conduit for the automatic dosage of the NaOH solution (6). The reactor was provided with an opening for hypochlorite proportioning and for analytic sampling (5). Inside of the reactor there were put 2.5 kg of starch milk of about 30% strength (1 kg of starch); pH was settled on the level of 8.5 with the help of a pH-meter with the device

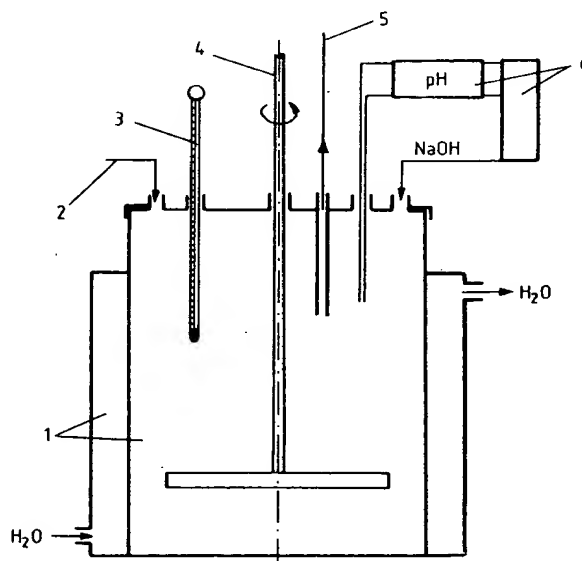


Figure 1. Reactor for starch oxidation by hypochlorite in water suspension; 1 — vessel with cooling jacket, 2 — reagents introduction, 3 — temperature measurement, 4 — stirrer, 5 — sampling, 6 — measurement and automatic pH control.

proportioning 20% of NaOH solution and automatically maintaining pH of reagents in the bounds of pH value $8.5 \div 9.0$; pH control during the oxidation process is necessary because with the course of reaction occurs constant depreciation of pH.

The reduction of available chlorine content in the reaction medium is the preliminary measure of the course of the oxidation process. Thus, in fixed intervals of time the liquid was sampled, consumption of available chlorine was checked and successive doses of hypochlorite were proportioned or, when the available chlorine was completely used up, the reaction was stopped.

When the oxidation process was completed, starch milk was unloaded from the reactor, starch was filtered off the reagent solution by glass filter. The oxidized starch was suspended in a quadruple quantity of water, next it was neutralized by HCl solution to pH = 6.5, the solution was filtered and the starch was washed with water until the reaction on chlorides decayed. Wet, oxidized starch was dried in a thin layer in the open air at room temperature to the humidity of about 20%. Obtained, in this way, the preparations of oxidized starch were stored and analyzed.

3.1 Influence of the Granularity of Potato Starch on the Course of its Oxidation in Water Suspension

One of the most important factors distinguishing potato starch from the other kinds of starches is the great differentiation of grain sizes. Potato starch consists of granules of $2 \div 110 \mu\text{m}$ sizes. The great differentiation of potato starch grains is not limited only to their sizes but it includes very essential differences of external and internal grain structure and even differences in the chemical composition.

As opposed to the big grains of enlarged capillary system, small granules most often possess a very compact structure with a very poor capillary system inside and are almost devoid of slits and ducts on the surface. On that score, they more approximate granules of some cereal starches.

Beside the noticeable physical differences of potato starch grains some examinations also show fundamental differences in chemical composition of large and small grains – the bigger content of phosphorus in starch of small grains was for the first time discovered by *Sroczyński* [18].

So essential physico-chemical differences among various potato starch grains must undoubtedly appear in the form of the differentiated behaviour of potato starch granules in technological processes and chemical reactions. It results at least from the differentiated surface of the contact of reagents with the carbohydrate mass of granules. All the observations let us assume that the determination of the influence of potato starch granularity on the course and the results of the reaction of oxidizing it by hypochlorite may be an important element in elucidating the process of potato starch oxidation. It is so essential because granularity of starches (or percent share of small and big grains in starch) coming from different regions of the country and from different factories is different. It results both from different conditions of starch biosynthesis and from some differences technically-technological of the particular potato starch works. These facts suggest a hypothesis that potato starch of different origin, and in the first place of different granularity, can behave otherwise in the process of the physico-chemical modification, that is also during oxidation.

In order to realize the research leading to the ascertainment of these differences, the sample of 50 kg commercial potato starch was divided into three fractions:

1. fraction of big grains ($> 50 \mu\text{m}$) – 5 kg;
2. fraction of medium grains ($20 - 50 \mu\text{m}$) – 20 kg;
3. fraction of small grains ($< 20 \mu\text{m}$) – 2 kg.

The division into fractions was done by the hydraulic classification method consisting in the slow flow of diluted (about 1%) water suspension of starch in six troughs placed in cascades. The hydraulic classification was carried out in that way that deposited, during the flow, starch fractions on the particular troughs (big grains undergo the sedimentation earlier and small ones flow down to the last trough) were collected periodically in separate tanks. The process of hydraulic classification of thus obtained fractions was repeated in succession until (about 50 times) the fraction of big grains was completely devoid of small and medium grains and the fraction of small grains was devoid of big and medium grains.

The fractional starch was dried in open air at a temperature of 20°C and was analyzed and used in experiments. Attempts of oxidation of fractional starch and of initial starch sample, at hypochlorite dose containing 4% of available chlorine, on starch were made.

The results of these experiments are given in Table 1. The setting up of the results show that the course and effects of potato starch oxidation depend, to a high degree, on its granularity. It also confirms the differentiation of phosphorus content in starch of big grains and small grains. There is no doubt that potato starch of big grains is more easily subjected to the process of oxidation than starch of small grains and after a short time of the reaction it gets a higher degree of oxidation than small grain starch. The regularity can be observed very well in the initial period of the course of the oxidation process. As the period of reaction is prolonged over 180 min, with use of a considerable dose of hypochlorite, then the destruction of big grains can be observed, and that causes flowing out of easily soluble fractions of strongly oxidized polysaccharides molecules (Table 2) from their inside.

The process of the progressive destruction of big grains can be observed during oxidation as well as during the analytical procedure and chiefly during sample washing with an acid for the displacement of cations neutralizing carboxyl groups of starch. It may happen that big grain starch when oxidized intensively and for a long time, gives the result of determination of carboxyl groups content smaller than analogous oxidized starch of small grains, that is caused by the loss of starch substance strongly oxidized by its flowing-out through slits of damaged and porous big grains, what is not observed in the case of small grains.

In order to confirm the fact, the results of the experiment consisting in long-lasting oxidizing of a big small grains fraction were mentioned (Table 2). The results show clearly that big grains are very quickly subjected to the action of the oxidizing agent in the initial period of the process. Small grains are oxidized with difficulty, slowly but after the prolonged action we observe in them the permanent increase of carboxyl groups content to the higher value in the final phase, than it takes place in the case of big grains and in an initial sample. It must be added, what results from the differentiated yield of granular product after 8 h of the process, that in the final phase (after 4 h) of lasting of oxidation process, serious big grains degradation can be observed (the decrease of the yield of granular product in the samples 1 and 2). This degradation, in the field of microscopic vision, is seen in the form of distinct fractures of grains and in the formation of breaches on the surface of big grains.

Table 1.

Influence of Potato Starch Granularity on the Course and Effects of Oxidation Process in Water Suspension; pH = 8 ÷ 9, milk strength 3%, hypochlorite dose 4% Cl on starch proportioned once, the temperature of the reaction 30°C (given results are the average of 3 parallel experiments).

Kind of starch	Time of reaction (min)	Viscosity of 1% sol. (mPa · s)	Content of groups (mmol/100 g)			
			carbonyl	acid in general	acid phosphate	carbocyl
Initial starch of granularity:	0	84.624	0.4	3.8	3.8	0
big grains -12%	30	7.686	1.6	10.5	3.2	7.3
medium grains -73%	60	2.564	4.8	15.3	2.8	12.5
small grains -15%	90	1.760	9.4	19.7	2.4	17.3
	120	1.654	11.2	22.5	2.2	10.3
	180	1.483	13.3	25.1	2.0	23.1
Large grains fraction (Ø = 50 ÷ 110 µm)	0	63.574	0.4	2.6	2.6	0
	30	6.394	1.4	13.0	2.5	10.5
	60	2.140	3.2	18.6	2.3	16.3
	90	1.913	8.8	22.1	2.0	20.1
	120	1.481	11.3	26.4	2.0	24.4
	180	1.394	13.6	28.2	1.7	26.5
Medium grains fraction (Ø = 20 ÷ 50 µm)	0	78.595	0.4	2.9	2.9	0
	30	7.846	1.5	7.9	2.8	5.1
	60	2.172	5.4	14.1	2.8	11.3
	90	1.857	9.2	17.5	2.7	14.8
	120	1.526	11.8	23.1	2.4	20.7
	180	1.491	14.1	26.5	2.2	24.3
Small grains fraction (Ø = 5 ÷ 20 µm)	0	87.697	0.3	4.1	4.3	0
	30	10.436	1.6	8.7	4.0	4.7
	60	9.375	4.9	13.2	3.7	9.5
	90	8.541	10.4	16.3	3.3	13.0
	120	6.815	12.6	17.3	3.2	16.1
	180	5.687	13.9	21.8	3.0	18.8

Table 2.

Course and Effects of the Prolonged Starch Oxidation in the Form of Large and Small Grains Fraction; pH = 8 ÷ 9, milk strength 30%, hypochlorite dose containing 10,0% Cl on starch proportioned in 5 parts, the temperature of the reaction 30°C

Kind of starch	Time of reaction (h)	Viscosity of 1% sol. (mPa · s)	Content of groups (mmol/100 g)				Product yield after 8 h of oxidation (%)
			carbonyl	acid in general	acid phosphate	carboxyl	
Initial starch of granularity:	0	83.650	0.35	3.7	3.7	0.0	
big grains -10%	1	8.360	8.4	11.8	3.4	8.4	
medium grains -68%	2	4.674	11.6	20.3	3.1	17.2	
small grains -22%	4	2.485	14.3	27.5	2.8	24.7	
	6	1.321	15.5	31.3	2.8	28.5	
	8	1.263	16.6	40.5	2.5	38.0	92.0
Big grains fraction (Ø = 50 ÷ 110 µm)	0	78.452	0.4	3.6	3.6	0.0	
	1	7.740	10.4	16.2	3.5	12.7	
	2	4.483	12.7	23.8	3.2	20.6	
	4	2.315	14.2	29.3	3.1	26.2	
	6	1.884	16.4	30.9	2.4	28.5	
	8	1.260	17.8	34.5	2.1	32.4	86.3
Small grains fraction (Ø = 5 ÷ 20 µm)	0	89.425	0.4	4.3	4.3	0.0	
	1	13.170	8.6	11.6	4.0	7.6	
	2	10.524	10.8	20.1	3.7	16.4	
	4	6.170	14.7	31.5	3.6	27.9	
	6	2.455	16.4	40.0	3.2	36.8	
	8	1.430	18.6	43.7	3.0	40.7	95.6

3.2 The Physico-chemical Changes of Starch Properties in the Oxidation Process

Five oxidized starch preparations in the best conditions, by the action of sodium hypochlorite in the water suspension of starch were the subject of physical and chemical examination (Table 3).

3.3 Photometric Measurements of the Changes of Iodine Bonds by Starch Solutions According to its Degree of Oxidation

On the base of the course of the curves (Fig. 2) it is seen that with the increase of starch oxidation degree it decreases its colour complexing power of iodine. This is indicated by

Table 3.

Statement of Main Qualities of Starch Preparations Subjected to Examinations of Physico-chemical Changes.

No.	Kind of starch		Content of carboxyl groups (mmol COOH/100 g)	Reducing value (mmol CHO/100 g)	Viscosity of 1% sol. (mPa · s)	Temperature of gelatinization (°C)
1	crude starch	0.0% Cl	0	0.4	84.62	59 – 74
2	oxidized	0.5% Cl	2.4	1.2	14.51	56 – 71
3	oxidized	1.0% Cl	3.9	4.5	6.84	54 – 70
4	oxidized	2.0% Cl	8.2	6.5	2.63	52 – 68
5	oxidized	3.0% Cl	12.5	10.8	1.82	50 – 67
6	oxidized	5.0% Cl	26.6	11.9	1.42	43 – 67

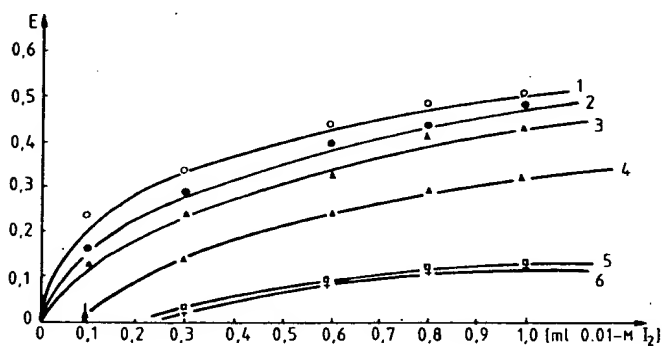


Figure 2. Change of absorbance (E) of iodine-starch complex solutions according to the participation of iodine at different degree of starch oxidation; 1 – crude starch, 2, 3, 4, 5, 6 – starches oxidized to different degree according to Table 3.

systematic increase of the inclination of the curves of absorbance value (E) together with increasing participation of iodine at the same content of starch mass. Also, together with decreasing colour intensity of starch-iodine complex there occurs, as the degree of starch oxidation increases, a change in the kind of colour from blue to violet. The changes of the colour complexing power of iodine by oxidized starches can be observed more clearly if colorimetric determinations of natural amylose content in examined oxidized starch samples during their storing in dry state are done. These changes are shown in Table 4.

The results in Table 4 show that starch oxidation causes a considerable reduction of natural amylose content of the spiral structure of the chain, that is to say, the complex forming power iodine-starch. At the same time, the ability grows stronger very gradually, as if it returns slowly, during the prolonged storage of oxidation products in dry state. The almost total decay of the ability for forming typical, blue colouring solutions of iodine-amylose complexes in strongly

oxidized starch and subsequent return of the colour is probably connected with the change of the form of starch molecules and not with the considerable degradation of amylose chains. As results from the measurements of molecular mass, the molecules remain big and hydrolysis of starch to the equivalent reducing value causes multiple lesser decrease of the coloured effects of iodine-amylose complexes and its return is not observed completely. For example, it is known that the change of colour of hydrolysate solutions with iodine shows a transition from blue colour to violet at the reducing value figuring about 20 DE which corresponds to about 100 mmol CHO/100 g. Meanwhile, the oxidized starch already at reducing value above 11 mmol CHO/100 g (2.0 DE) begins to show the change of blue colour into violet and the decay of blue colour and the transition into pure violet take place in an oxidized starch sample of the reducing value above 15 mmol CHO/100 g, that is about (3 DE).

3.4 Determination of the Carboxyl Groups Type in Starch Oxidized with Hypochlorite

Determination of carboxyl groups type constituting so called general reducing value of oxidized starch has fundamental meaning for the characteristics of the mechanism of starch oxidation. It may explain many author's contradictions or doubts with regard to the problem: are carboxyl groups in oxidized starch the products of oxidation reaction or are they the product of hydrolysis reaction of glucosides bonds accompanying the oxidation process? The explanation of this question is possible only by way of an unmistakable statement, whether the typical aldehyde groups occur in starch oxidized by hypochlorite, such as in dialdehyde starch, or whether the reducing value of oxidized starch originates only from liberated hemiacetal groups at anomer carbons of initial glucose residues. To explain this problem the test of absorption measurements of the infrared radiation (IR) by

Table 4.

Changes of Natural Amylose Content in Oxidized Starch Samples during their Storage; as a standard for determinations was used amylose preparation produced by Koch-Light Laboratories Ltd., special photometer $\lambda = 580$ nm, starch concentration in the colorimetric analyzed solution $1 \div 5$ mg on 100 ml.

No.	Starch oxidation degree (mmol COOH/100 g)		Kind of colour from I ₂	Content of amylose in starch samples stored in dry state (%)			
				when fresh	after a year	after 2 years	after 3 years
1	crude	0.0	blue	24.6	23.7	23.1	22.5
2	oxidized	2.4	blue	20.2	22.8	23.5	23.6
3	oxidized	3.9	blue	18.4	20.6	21.4	22.0
4	oxidized	8.2	blue	15.5	18.5	20.3	20.8
5	oxidized	12.5	blue-violet	9.4	14.8	17.0	18.2
6	oxidized	26.6	violet	3.6	10.4	13.2	14.5

the film made from the mixture of tested starch in paraffin oil in Perkin-Elmer's infrared spectrograph, was done. However, the results of these measurements appeared to be negative.

Numerous attempts of the modification of IR-spectrum measurement also did not permit any discrimination of the progress of starch oxidation in the range of carbonyl groups. On this base the test was acknowledged to be negative and useless for the characteristics of carbonyl groups in starch.

It was also tried to adapt a well-known qualitative reaction of aldehydes with *Schiff's* reagent to colorimetric quantitative analysis. It appeared that at the definite strength of fuchsin in acid medium, fuchsin-sulfurous acid gives positive colour reaction with some aldehydes, e.g. with formaldehyde, acetic, glyceric *etc.*, as well as with dialdehyde starch and at the same time it does not give a colour reaction with reducing sugars. To make sure of the specificity of the reaction, a lot of tests of quantitative determination of aldehyde groups content in the solution of starch oxidized by hypochlorite was made, using an analytical curve of absorbance dependence (E) from aldehyde content, verified in the presence of glucose and low-saccharified starch hydrolysates. The same determinations in dialdehyde starch samples of different degree of oxidation by sodium periodate were also done. The results of these tests are shown in Table 5.

It is seen from the results of colorimetric measurements that all the starch samples oxidized with hypochlorite give a negative reaction with *Schiff's* reagent while the dialdehyde starch formed in the result of specific oxidation by periodate gives very intensive violet-blue colouring. So it can be stated with a good deal of probability that the general reducing value of starch oxidized with hypochlorite is caused exclusively by reducing groups of initial glucose elements at the anomeric carbons in the chains of amylose and amylopectin molecules. Those samples lack typical aldehyde groups which unmask themselves clearly in dialdehyde starch. Samples of dialdehyde starch contain aldehyde groups being, however, only a small part of the general reducing value. So one must put up with the fact that the process and the reactions of starch oxidation are always accompanied by an intensive hydrolysis process of glycosidic linkages leading to considerable degradation of starch polysaccharide molecules, shown by the increase of reducing value and the decrease of the viscosity of the oxidation product. The reducing value of starch oxidized by hypochlorite cannot be the only measure of the degree of starch oxidation and also it cannot be included in the reaction balance of oxidation

Table 5.

Content of Aldehyde Groups in Oxidized Starch Determined Colorimetrically with *Schiff's* Reagent.

No.	Kind of starch (mmol COOH/100 g)		General reducing value	Content of aldehyde groups
			(mmol CHO/100 g)	
1	crude starch	—	0.4	0.00
2	oxidized with hypochlorite	2.4	1.2	0.00
3	oxidized with hypochlorite	3.9	4.5	0.00
4	oxidized with hypochlorite	8.2	6.5	0.00
5	oxidized with hypochlorite	12.5	10.8	0.00
6	oxidized with hypochlorite	26.6	11.9	traces of colour
7	dialdehyde	—	11.2	1.10
8	dialdehyde	—	18.4	3.45
9	dialdehyde	—	34.6	10.62

although there is the apparent increase of carbonyl groups; as those groups are formed not in the result of oxidation of hydroxyl groups but exclusively by the liberation of reducing groups in the hydrolysis process of glycosidic linkages.

3.5 Analysis of the Acid Products of Starch Oxidation

To do the quantitative and qualitative chromatographic analysis of acids formed in the result of oxidation, oxidized starch samples were subjected to the following processes in succession:

- 1) acid hydrolysis,
- 2) eduction (separation) of acids from starch hydrolysates with the help of mixed ion-exchanger bed.

The results of these analyses are shown in Table 6.

The balance of two determined acids — glucuronic and gluconic — with the general content of carboxyl groups in oxidized starch (Tab. 6) shows that the main attack of oxygen in starch molecules is pointed at the sixth carbon of the glucose element (glucuronic acid), and only to a low degree at anomeric carbons (gluconic acid) liberating themselves. These two acids, in principle, exhaust the whole state of carboxyl group content in starch oxidized with hypochlorite.

3.6 Enzymatic Saccharification of Oxidized Starch

Already during preparations for the chemical analysis of starch hydrolysates it has been found that there are very

Table 6.

Content of Glucuronic and Gluconic Acids in Samples of Oxidized Starch Hydrolysates.

No.	Kind of starch	Content of carboxyl groups	D. E. of hydrolyzate	Content of acids				
				Gluconic		Glucuronic		
				(mmol/100 g)	(% COOH)	Det. by chromatographic method	Det. by <i>Schoorl's</i> method	average
						(mmol/100 g)	(% COOH)	(% COOH)
1	crude starch	0	98.6	0	0	0	0	0
2	oxidized	2.4	97.4	0.63	26.2	1.81	1.76	74.8
3	oxidized	3.9	97.2	0.00	25.4	3.01	2.89	75.6
4	oxidized	8.2	97.3	1.98	24.1	6.35	6.02	76.2
5	oxidized	12.5	96.7	2.85	22.8	9.86	9.51	77.4
6	oxidized	26.6	97.1	6.14	23.1	20.38	20.35	76.5

serious difficulties in realisation of enzymatic hydrolysis of oxidized starch. Similar difficulties are signaled in Graefe's [7] and Fischer and Piller's papers [5].

Graefe, subjecting to enzymatic saccharification strongly oxidized starch with concentrated nitric acid, in order to obtain glucuronic acid, stated that such oxidized starch cannot be at all hydrolyzed by means of amylolytic enzymes but only with the help of acids. Because the difficulties occurring in the process of enzymatic hydrolysis of oxidized starch can have practical significance and cause definite technological difficulties, it was decided to check the influence of the oxidation process on the course of the enzymatic saccharification of starch.

The results of these experiments were collected in Table 7 and illustrated by means of a chromatogram in Figure 3.

The course of the process of the enzymatic saccharification of starch oxidized up to different degree indicates that indeed, oxidized starch yields the action of glucoamylase with greater difficulty. Even not highly oxidized samples, which do not yet show the presence of carboxyl groups and are only characterized by a small increase of reducing value and a large decrease of viscosity, are subjected to the process of enzymatic saccharification clearly more slowly. The final result of the saccharification process of oxidized starches is also lower than the reducing value of crude starch hydrolysate, at the same time the final degree of saccharification of oxidized starch is inversely proportional to the degree of the oxidation of hydrolyzed starch.

Paper chromatogram of oxidized starch hydrolysates (Fig. 3) shows that in the samples of oxidized starch hydrolysates spots of several higher oligosaccharides can be found, and also the sample of the eluate from the ion-exchange column gives, beside the traces of glucuronic acid, the same spots of oligosaccharides. This proves that these oligosaccharides contain carboxyl groups, and so the main difficulty in the process of enzymatic fission of the glucosidic linkages appears near the places of carboxyl groups occurrence in polysaccharides molecules of oxidized starch.

3.7 Capacity of Oxidized Starch for Forming a Flexible Gel Film

Application of oxidized starch as a preparation serving for pasting the warp in the weaving process, became prevalent in

Table 7.

Course of Enzymatic Hydrolysis of Oxidized Starch; liquefaction by α -amylase Novo 264; saccharification by glucoamylase Novo 150; concentration 30%.

Time of saccharification	Reducing value (DE) of oxidized starch hydrolysates containing carboxyl group (mmol/100 g)								
	Crude starch	Weakly oxidized			2.4	3.9	8.2	12.5	26.6
		0.0 ¹⁾	0.0 ¹⁾						
0	0.060	0.12	0.18	0.22	0.81	1.17	1.94	2.14	
after liquefaction	29.4	27.8	27.9	27.1	26.5	26.1	26.3	28.8	
6	56.2	49.8	48.5	49.4	48.2	47.0	46.6	45.9	
10	74.5	68.3	66.2	67.3	69.1	65.7	66.3	65.7	
20	78.7	71.2	70.6	71.8	70.3	67.8	68.2	66.5	
30	86.3	79.3	81.5	82.4	80.9	78.5	79.1	77.0	
40	97.1	89.7	86.2	89.9	83.7	79.8	79.7	79.9	
60	98.3	96.9	96.5	94.6	86.5	80.4	81.4	81.6	
80	98.8	97.8	97.3	94.9	89.3	96.1	84.3	82.6	
100	97.9	97.9	97.1	94.3	88.6	86.8	85.1	82.5	

¹⁾ Samples of weakly oxidized starch, because of that carboxyl groups content is not perceptible analytically and only decreased viscosities from 84.6 mPa · s to the value in succession 21.4 mPa · s and 14.3 mPa · s can be determined.

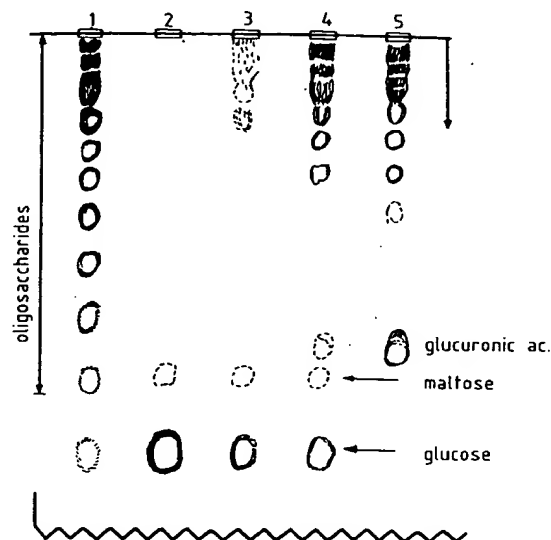


Figure 3. Reproduction of paper chromatogram of enzymatic hydrolysates of oxidized starch; 1 - liquefied enzymatically crude starch, 2 - saccharified crude starch (α -amylase + glucoamylase), 3 - 4 - saccharified oxidized starch (2.4 and 26.6 mmol COOH/100 g), 5 - eluate from ionites of enzymatic hydrolysate of oxidized starch.

the result of practical observation of the behaviour of different starch covers (apprets) on fabrics. It has turned out that appret preparations obtained from modified starch and particularly from oxidized starch are characterized by greater elasticity of cover film and by greater adhesion to fibres and paper than the preparations from crude starch, or its hydrolysates.

The capacity of oxidized starch for forming elastic gels probably results from its greater possibility of forming systematic structures of cross-linked gel compared to crude starch. Such structures form relatively easily straightened chains of oxidized amylose likened in shape to cellulose in comparison with the disordered spatial structure of spiral balls of natural amylose.

On the base of practical settlements and observations the attempt of obtaining elastic gel from glycerol and sorbitol solutions of oxidized starch was carried out. When it was

checked that gels obtained from oxidized starch possess the properties of flexible material, films were made from these solutions.

Obtaining of starch films was effected by pouring the starch solutions on the surface of a teflon plate and separating a flexible gel film after the evaporation of the excess water at room temperature (exiccation).

Examinations of mechanical properties of the obtained starch films showed that:

- 1) starch oxidized from hypochlorite up to the content of at least 20 mmol COOH/100 g, an edible film of great flexibility and transparency can be obtained (similar to sheeting polyethylene) preserving its qualities for the period of about a year; in this period the starch film loses its flexibility and becomes brittle and that is probably connected with the inversion of changes in time (Table 4);
- 2) under the same conditions crude starch and little oxidized starch form a brittle film, opaque to such a degree that in consequence of the great brittleness it cannot be separated from a teflon plate without damage.

As a result of the experiments the production technology of obtaining edible starch films for packaging of selected food products was worked out [2].

4 Conclusions

Oxidation of potato starch with hypochlorite causes the following physico-chemical changes:

1. starch grains are subjected to damages which consist in cracks in the centre and partial liquefaction; when advanced, the damages are particularly visible in large grains;
2. lamination and micellar structure of grains in oxidation process stay untouched;
3. in the result of oxidation, in starch molecules carboxyl groups at carbons 6 and 1 (glucuronic acid about 75%, gluconic acid about 25%) are formed;
4. hydrolysis of glucoside linkages is the side reaction which takes place during oxidation, in the result of which there is the increase of starch reducing value and the decrease of viscosity;
5. in alkaline medium there no dialdehyde systems are formed in starch molecules and the whole reducing value

comes from carboxyl groups situated at anomer carbons of glucoside elements;

6. the change in the range of the secondary molecular structure (spatial location of molecules) is the most essential. These are the results of this transformation:
 - decrease, up to decay, of the colour effect of amylose — iodic complexes;
 - increase of resistance of oxidized starch to the action of saccharifying enzymes;
 - appearance of the capacity of oxidized starch for forming flexible gels (films) and clear solutions.

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